

## **Detection (FISH)**

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### **Introduction**

Probes labeled with biotin must be detected with fluorescently labeled Avidin, and probes labeled with digoxigenin require detection with a fluorochrome conjugated antibody against this hapten. For example, to detect the biotin labeled probes we routinely use Avidin-FITC, Avidin-TRITC, or Avidin Cy-5. For the probes labeled with digoxigenin, we usually first incubate with mouse-anti-digoxigenin, followed by incubation with sheep anti-mouse Cy5.5, or other fluorochrome conjugated antibodies.

### **Reagents**

#### **Avidin-Cy5**

Jackson Immuno Research Lab, Cat. 003-170-083

#### **Avidin-TRITC**

Sigma, Cat. A 7169

#### **Avidin-FITC**

Vector, Cat. A-2011

#### **BSA (Bovine Serum Albumin)**

#### **DAPI**

#### **Ethanol, absolute**

#### **Formamide**

Fluka BioChemika, Cat. 47671

#### **HCl, 1N**

#### **Mouse anti-digoxigenin**

Sigma, Cat. D 8156

#### **Sheep anti-mouse Cy5.5**

Amersham, Cat. RPQ 0115

#### **20X SSC**

#### **Tween 20**

## Preparation of Reagents

### 50% FA/SSC

20X SSC                      30 ml  
dH<sub>2</sub>O                        120 ml  
Formamide                  150 ml  
Adjust pH to 7 with 1N HCl

**Pre-warm to 45°C**

### 1X SSC (for direct labeled probes, i.e., TRITC, FITC or other)

20X SSC                      25 ml  
dH<sub>2</sub>O                        475 ml

**Pre-warm to 45°C**

### 0.1X SSC (for indirect labeled probes, i.e. Biotin, or Digoxigenin)

20X SSC                      2.5 ml  
dH<sub>2</sub>O                        497.5 ml

**Pre-warm to 60°C**

### 4X SSC/0.1%Tween20

20X SSC                      200 ml  
dH<sub>2</sub>O                        799 ml  
Tween 20                      1 ml

**Pre-warm to 45°C**

### Blocking Solution ( 3% BSA/4X SSC/0.1%Tween20 )

BSA                              0.3 g  
4X SSC/0.1%Tween 20      10 ml

**Pre-warm to 37°C**

### Antibody Solution ( 1% BSA/4X SSC/0.1%Tween 20 )

BSA                              0.1 g  
4X SSC/0.1%Tween 20      10 ml

**Pre-warm to 37°C**

### DAPI stock solution (f.c.= 0.2 mg/ml)

DAPI                            2 mg  
ddH<sub>2</sub>O                        10 ml

Aliquot and store at -80°C

### DAPI staining solution (f.c.= 80 ng/ml)

DAPI (stock solution)      40 µl  
2X SSC                        100 ml

Store at 4°C in a light-tight coplin jar

## Procedure

1. Carefully remove the rubber cement surrounding the coverslips from hybridized slides.
2. Wash the slides in 50% formamide/2X SSC (pH 7-7.5) for 3 x 5 min at 45°C, shaking.
3. Wash slides in 0.1X SSC at 60°C (for indirectly labeled probes) or 1X SSC at 45°C (for directly labeled probes) for 3 x 5 min, shaking.
4. Dip slides in 4X SSC/0.1%Tween 20.
5. Add 120 µl of Blocking Solution (3% BSA/4X SSC/0.1%Tween 20) to the slides and cover them with a 24 mm x 60 mm coverslip in a moist hybridization chamber at 37°C for 30 min.
6. Dip slides in 4X SSC/0.15%Tween 20 to wash off the blocking solution. Proceed directly to step 9 if using a directly-labeled probe.
7. For indirectly-labeled probes (Biotin or Digoxigenin), add 120 µl of fluorescent antibody (antibody should be diluted 1:200 in 1% BSA/4X SSC/0.1%Tween 20) to the slides, cover with a 24 mm x 60 mm coverslip, and incubate in moist light-tight hybridization chamber at 37°C for 45 min.
8. Wash slides in 4X SSC/0.1%Tween 20, for 3 x 5 min, shaking.
9. Stain slides for 5 min in DAPI staining solution in a light-protected coplin jar.
10. Wash the slides for 5 min in 2X SSC, shaking.
11. Dehydrate the slides by dipping through an ethanol series of: 70%, 90%, and 100%; air-dry.
12. Apply 35 µl of antifade solution, cover with 24 mm x 60 mm coverslips, store in light-protected container at 4°C until slide is imaged.

## Notes

1. Exposure of slides to ambient light should be minimized during all procedures.

2. Use care in removing coverslips during all procedures to minimize scratches.
3. Spin all fluorescent dyes prior to use for 3 min at 13,000 rpm and carefully pipette the antibody without disturbing the pellet.
4. Do not let the slide dry out between washing steps.